Screening and Authentication of Mineral Precipitating Bacterial Isolates for the Formation of Tangible Biomaterial for Microbial Concrete

Synopsis
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CERTIFICATE

It is certified that Mrs.Ujjwala Rani is submitting her synopsis for Ph.D. under my guidance on the Topic "**Screening and Authentication of Mineral Precipitating Bacterial Isolates for the Formation of Tangible Biomaterial for Microbial Concrete***"*.

She has prepared this synopsis under my guidance and supervision and I have gone through the content presented in this report.

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Abstract

There are different organism that exhibits on earth that includes plants, animals and microorganisms. All of them, micro-organisms are most primitive. Bacteria are microscopic unicellular organisms that are prokaryotic in nature. Bacteria can thrive in different environment conditions. Some of them can survive in extreme high temperature to high alkalinity conditions. Many bacteria have some special mechanism of mineral precipitation that may help them in survival of that particular habitat. This phenomenon of precipitation of mineral by bacteria is known as bacterial mineral precipitation. Bio mineralization may have sustainable application like bacteria can be used as bio-concrete; it may help in extraction of some valuable minerals. These processes also have a very good application on restoration of surfaces. This study mainly focuses on the screening and exploiting this tendency of bacteria for their mineral precipitation. For this research, bacterial sample will be collected from specific site and screened for the potency of mineral precipitation with selective parameters and used for the preparation of biomaterials.

Key words: Bacteria, mineral precipitation, biomaterial

Introduction

Microorganisms are ubiquitous in nature. Microorganism are diverse consist of algae, bacteria, protozoa, fungi, viruses etc. In the evolution process; microorganisms have adapted to their habitat almost every corner of the world. We can found them in human body to extreme environmental conditions like volcanoes to icebergs. Usually microorganisms belong to prokaryotes. They exhibit different characteristics depending on the extrinsic and intrinsic factor (Saldana-Vazquez *et al.*, 2013).

Bacteria are single cell, microscopic, prokaryotic microorganism. They are simple cellular structure having primitive nucleus. Bacteria have special characteristics like they can survive on organic as well inorganic sources as food. Some bacteria are harmful for us but some plays an important role in biogeochemical cycles, industrially important and many of them has calcification phenomenon.

In natural environmental conditions, biomineralization is a well-known phenomenon leading to the formation of more than sixty different types of biological minerals (Sarikaya, 1999) that exists as extracellular inorganic (Dhami *et al.*, 2013) or intracellular crystals (Konishi *et al.*, 2006 and Yoshida *et al*., 2010). Microbial biomineralization is the process of formation and deposition of minerals directly mediated or indirectly influenced by microorganisms (Mann, 2001).

Bacteria may form mineralized crystals within the cytoplasm or in the cellular membrane that varies according to various factors as well as species to species. It plays a crucial role in the cementation of various natural substrates like coral such as ancient caves, sediments, and oceans areas. They are able to precipitate a wide range of minerals that include: phosphates, carbonates, sulphates, chlorides, oxalates and silicates (Riding, 2000; Banks *et al*., 2010 and Chou *et al*., 2011).Taking this as a research opportunity we can exploit these characteristics

of bacteria as tools for extraction of valuable minerals like gold, titanium, for restoration work and many more.

Review of Literature

Bacteria play a very crucial role in our life. Some bacteria are beneficial and vice-versa. Mineral precipitation may occur as a result of microbial metabolic activities like ureolysis, sulphate reduction, denitrification and methane oxidation. The extracellular polymeric substrate may act as base templates affecting calcium carbonate crystal morphology, spatial position, polymorphism, and growth. (Tourney and Ngwenya, 2009; Oppenheimer-Shaanan *et al.*, 2016; Braissant *et al.*, 2003 and Ercole *et al*., 2012). In the process of mineral precipitation where the concentration of calcium was higher in habitat, the polymorphs produced mainly are calcite, aragonite and vaterite depending upon both types of bacteria as well as on environmental conditions (Alonso *et al*., 2019).

Mineralization by bacteria was broadly classified into two classes i.e. Biologically controlled mineralization (BCM) and Biologically induced mineralization (BIM) (Lowenstam and Weiner, 1989). In case of BCM under certain conditions minerals may directly synthesized at nucleation site within or on the cell. In case of biologically induced mineralization, continuous metabolic activity of the organism which results in the precipitation of mineral that occurred extracellularly. This extracellular production of these biominerals opens a new research concept globally for exploiting this ability of microbes for various bioengineering and biotechnological applications. From all the different variety of minerals associated with biomineralization, carbonates are the widely studied (Dhami *et al.*, 2013).

Bacterial Calcium Carbonate Mineralization (BCCM) is a common natural phenomenon usually occur in various bacterial taxonomic groups in different environmental conditions which effects the formation of microscopic crystals to large geological formations (Boquet *et al*., 1973; Ehrlich, 2002; Dupraz *et al*., 2009; Perito and Mastromei, 2011). Alkaline environment may encourage the bacterial growth and increases the level of dissolved

inorganic carbon through different metabolic pathways (Dhami *et al*., 2014; Zhu and Dittrich, 2016).

The main benefit of a BCCM mediated treatment is that it imitates the natural process responsible for producing a mineral product similar to the stone substrate. The goal was to provide a consistent $CaCO_3$ layer on the surface of deteriorated substrate, protecting against the chemical, water intake and to strengthen the inner, weakened structure (De Muynck*et al*., 2013).

Microbially induced calcium carbonate precipitation (MICCP), expansively studied section of biomineralization which has many application in different fields like biotechnology to civil engineering (Marques *et al.*, 2020). It is a biochemical process in soils, caves, freshwater, marine sediments, and hyper saline habitats. Bio-concrete production is based on the addition of bacteria with the ability to induce the formation of minerals (biomineralization) from the cement matrix. This process is facilitated through the ingress of water, CO_2 and other chemical substances such as SO_4 and NO^{-3}, among others (De Muynck *et al.*, 2008; Jonkers *et al.*, 2010; Dhami *et al.*, 2014; Achal and Mukherjee, 2015; Bundur *et al*., 2015; Verma *et al*., 2015), aids in self-healing and improves the physical and mechanical properties of the concrete structure.

Some of the common bacteria found in soil is *S. pasteurii* that may be used in many processes like remediation, heavy metal contamination, concrete remediation and soil improvement (Li, Chen and Burne, 2013; Phillips *et al.*, 2013b; Whiffin *et al*, 2007), whereas *B. megaterium* used to enhance concrete hardness and construction material durability (Dhami *et al*., 2014; Soon *et al*., 2013).

Of late, several reviews have been published on biotechnological aspects of bioconcrete (Zhu and Dittrich, 2016; Joshi *et al*., 2017; Krajewska, 2018; Jain and Arnepalli, 2019), but microbiological and molecular concepts of MICP and the mechanisms of MICP by different microbial groups have not yet been reported (Alonso *et al*., 2019).

Mineral precipitation tendency of bacteria can be used in the formulation of biocement, to treat deteriorated surfaces or to enhance the compressive strength of cement. Biocement (microbial concrete) has many possible applications like use as a potential sustainable design material, based on its 40% compressive strength compared to conventional concrete, improved finishing, and environmental impact (Lee *et al*., 2018).

1. **DESCRIPTION OF BROAD AREA/ TOPIC**

   This research is proposed on the concept to utilize the mineral precipitation tendency of the bacteria which can be used in applied microbiology. Some of the area includes precipitation of polymorphs of calcium which may be used as restorative agent. It can also use as for the formation of tangible bio-materials with potent bacterial isolates.

2. **OBJECTIVES OF THE STUDY**

   A. To collect the samples from marble industry (slurry) and Sambhar lake (soil) and analysis of physico-chemical property of collected samples.

   B. To identify various bacterial isolates on the basis of morphological and biochemical characterization.

   C. To screen and selecting the potential bacterial isolate for mineral precipitation by using Microscopic techniques.

   D. To optimize the conditions on the basis of pH, solid and liquid medium for minerals precipitation.

   E. To authenticate the selected isolates using modern tools for molecular characterization.

   F. To analyse potent isolates as tangible biomaterial preparation in bench scale study.

3. **METHODOLOGY TO BE ADOPTED**

A. To collect the samples from marble industry (slurry) and Sambhar lake (soil) and analysis of physico-chemical property of collected samples.

This study is focusing on isolating heterotrophic bacteria from different top soil collected with a view to understand the diverse microbial flora and their potential to mineral precipitation. Our main sample collection site would be the drainage area of marble factory from Jaipur and Sambhar lake due to high alkaline condition helps microorganism in the mineral precipitation.

Sampling

The slurry samples used for this work will be collected from two different locations i.e drainage outlet of marble factory and Sambhar Lake. These samples will be labelled according to the site of collection as MF and Sambhar Lake as SL under precautionary measures.

Theses sample are also analysed for their physico-chemical parameters which are as follows: temperature, pH, hardness, total dissolved solids, alkalinity, turbidity, mineral like Calcium, Chloride, bicarbonates.

B. To identify various bacterial isolates on the basis of morphological and biochemical characterization.

i. Morphological and Cultural Characterisation:

Classical microbiology uses both gross and microscopic morphology to identify microbes. Gross morphology includes whole shape of colony, colony size, colony edges/margins, pigmentation and surface of colony, consistency or texture of colony.

After the isolation of pure culture by streak plate method further identification can be done with simple staining and Grams staining etc. and structure of bacteria will be analysed using microscopic technique.

ii. For Biochemical Characterisation:

For identification of bacterial isolates and in the identification of different bacterial species, biochemical reactions plays are very important role. These biochemical tests

depend on the presence of certain enzymes, such as catalase, oxidase, urease, gelatinase, etc., produced by the bacteria. This proposed study includes a series to biochemical test that includes urease test, catalase test, oxidase test, sulphur reduction test, nitrate reduction test and sugar fermentation test.

Some of the brief biochemical test for characterization of bacteria is as follows Chauhan and Jindal, (2020).

Catalase test:

Catalase test is used to detect the presence of the enzyme catalase which is found in most bacteria including most of aerobic and facultative anaerobic bacteria. Catalase is not found to be present in anaerobes. This enzyme is responsible for breakdown of hydrogen peroxide (H_2O_2) with the release of free Oxygen.

Oxidase test:

Oxidase test is used to determine the presence of oxidase enzymes in the bacteria. In this test, the reagent contains tetra methyl-p-phenylene diamine dihydrochloride that serves as an alternative substrate for the cytochrome oxidase reaction. In the reduced state, this reagent is colourless but when oxidized, it becomes purple. That helps in identification that bacteria is able to produce oxidase enzyme.

Urease test:

Urease test is very important for this proposed research because it used as a base test to differentiate bacteria for mineral precipitation. It determines the ability of bacteria to hydrolyse urea to make ammonia using the enzyme urease. For this, urease broth is used which specifically contains urea and an indicator i.e. phenol red. This phenol indicator turns yellow in an acidic environment and red in an alkaline environment. If the urea in the broth is degraded and ammonia is produced, an alkaline environment is created, and the media turns red. This test helps to separate species of the genus *Proteus* from the urease-negative *Salmonella* and *Shigella* species.

IMViC test: Indole, Methy red, Voges-Proskauer, Citrate utilization test

Indole test: For this test tryptone water inoculated with isolates. Incubated at 37°C for 24 hrs. After incubation, a few drops of the kovac's reagent will be added. Based on red-coloured ring formation, bacteria are distinguished as indole positive or negative.

Methyl red test: In this test MR-VP Broth inoculated with the isolates and incubated at 37°C for 24 hrs. After incubation, a few drops of methyl red indicator solution will be added to the tubes. Based on colour change to cherry red of MR-VP broth, bacteria are distinguished as Methyl Red (MR) positive or negative.

Voges-Proskauer test: For this test, MR-VP Broth inoculated with the isolates and incubated for 24 hour. After incubation, 0.5 ml α-naphthol (Baritt A reagent) solution and 0.5 ml 40% KOH solution containing 0.3% creatine (Baritt B reagent) will be added to the tubes. Based on colour change to red of MR-VP broth, bacteria are distinguished as VP positive or negative.

Citrate utilization test: In this test**,** slants of Simmon's Citrate agar was streaked with the isolates and incubated at 37°C for 48 hours. Due to rise in pH of the medium from its initial green colour to deep blue. Based on colour change to deep blue, results are reported as citrate positive or negative.

Nitrate reduction test:

This is an important test to determine the ability of an organism to reduce nitrate to nitrites. Production of red colour occurs in the presence of nitrite and indicates the ability of the organism to reduce nitrate to nitrite.

Triple sugar iron agar (TSI) test:

TSI Agar is used to differentiate gram-negative enteric bacilli based on carbohydrate fermentation and the production of hydrogen sulphide. This test is determine the ability of an organism to attack a specific carbohydrate incorporated into a basal growth medium by fermentation of glucose, lactose and sucrose present in it along with the determination of hydrogen sulphide (H_2S) production.

A pH indicator changes the colour of the media in response to fermentation. The difference in colour of the media in response to fermentation indicates which sugar is fermented.

Sometime, presence of a black colour indicates that H_2S was produced. In this media, H_2S reacts with the ferrous sulphate and convert it into ferrous sulphide that gives black colour.

C. To screen and selecting the potential bacterial isolate for mineral precipitation by using Microscopic techniques.

After biochemical characterizations, selective bacterial isolates will be further screened out for the potency of mineral precipitation on B4 medium. These isolates will be regularly checked out at defined time using Phase contrast microscope. These will be regularly observed for seven day leads to differentiate between mineral precipitating bacteria and non-mineral precipitating bacteria. All the plates will be further examined using microscopy to check the formation of crystals and colonies will be counted. After this, these isolates will further purified by re-streaking to isolate pure colonies.

D. To optimize the conditions on the basis of pH, solid and liquid medium for minerals precipitation.

For optimization of pH, selected isolates will be grow at different pH, bacterial strains will be inoculated on modified B4 solid plates of different pH and will be incubated under aerobic conditions at 35-39◦C temperature (Marvasi *et al*.,2012).

For optimization of liquid and solid media on the basis of percentage of calcium acetate on optimum pH and temperature.

For the optimization of temperature will be done on the basis of different range of temperature on optimum pH and optimum media concentration. Regularly at defined time slot, any change in crystal formation will be checked using SEM.

E. To authenticate the selected isolates using modern tools for molecular characterization.

Past decade showed that the molecular microbiology methods mark a revolutionary impact in the bacterial identification process. It is a rapid and accurate but also very precise and widely used by the researchers. Molecular tools allow us to identify the strains which may sometime very much difficult to culture in-vitro conditions. In bacteria, the genes which encode the 16S rRNA are highly conserved because it has an important function in ribosome assembly. Because of these characteristics, the 16S rRNA gene has become an ideal gene fragment to

be used for identification, comparative study and phylogenetic studies and classification of bacteria.

Phylogentic analysis:

Phylogentic analysis steps would be done after 16s rRNA gene sequencing. In this process, the output nucleotide sequence will be forwarded to bioinformatics tool i.e. n-BLAST Analysis process. The website that is going to be used is http://blast.ncbi.nlm.nih.gov in NCBI. Further this nucleotide sequence will be deposited in Genbank database for a unique universal accession number. By using the desired nucleotide query sequence and control sequence a phylogenetic tree will be constructed using Molecular Evolutionary Genetics Analysis Tool (MEGA 5.1) (Tamura *et al*., 2013).

Based on the similarity of query sequence (16s rRNA gene sequence obtained) after BLASTn the exact species/strains of bacteria will be reported. A phylogenetic tree will help to assess the evolutionary history and close relatives.

F. To analyse potent isolates as tangible biomaterial preparation in bench scale study.

For analysis of isolates as restorative tool and tangible material small bench scale experiments will be set.

- In first experiment, deteriorated stone will be collected form the marble site and will be smeared with consortium isolates. Comparison of SEM images will be checked the restoration potential of biomineralizing bacteria (Sazanova *et al*., 2020).
- In second experiment, different potent bacterial isolates cell concentrations will be formulated with cement to check the formation of tangible biomaterial. Tangibility of bio-cement will be further characterized on the basis of its water absorption and compressive strength (Seifan *et al*, 2016, Mondal and Gosh, 2018).

PROPOSED / EXPECTED OUTCOME OF THE RESEARCH

This research work helps us to understand the role of bacteria in mineral precipitation phenomenon which has many potential applications. Non- metallic minerals like limestone, granite, phosphates of calcium, polymorphs of calcium have industrial importance. Our approaches is to give an sustainable resource that may help in extraction of these minerals as well as some of them may acts as bio-paint for restoration of deteriorated surfaces as well as help in formation of more tangible biomaterial as eco-friendly approach towards the environment.

References:

1. Abo-El-Enein, S.A., Ali H.A, Talkhan F.N. and Abdel-Gawwad,H.A.(2013) 'Application of microbial biocementation to improve the physico-mechanical properties of cement mortar', *Housing and Building National Research CenterJournal,* 9 (1),pp.36-40. DOI: https://doi.org/10.1016/j.hbrcj.2012.10.004.
2. Alonso,M.J.C., Hernandez, L.E.M., Munoz, M.A.S., Franco M.R.M., Narayanasamay,R., and Balagurusamy,N. (2019) 'Microbially Induced Calcium Carbonate Precipitation (MICP)and Its Potential in Bioconrete: Microbiological and Molecular Concepts', *Frontiers In Materials* 6, pp.126-141. DOI: https://doi.org/10.3389/fmats.2019.00126.
3. Anbu, P., Kang, C.H., Shin, Y.J. and So, J.S. (2016) 'Formations of calcium carbonate minerals by bacteria and its multiple applications', *Springer Plus,* 5**(1)**,250 DOI: https://doi.org/10.1186/s40064-016-1869-2.
4. Banks, E. D., Taylor, N. M., Gulley, J., Lubbers, B. R., Giarrizo, J. G., Bullen, H. A., Hoehler, T. M. and Barton, H.A. (2010) 'Bacterial calcium carbonate precipitation in cave environments: a function of calcium homeostasis', *Geomicrobiology Journal,*27(5), 444–454. DOI: 10.1080/01490450903485136.
5. Berdoulay, M. And Salvado, J.C. (2009) 'Genetic characterization of microbial communities living at the surface of building stones', *Letters in Applied Microbiology,* 49 (3),pp. 311–316. DOI:10.1111/j.1472-765X.2009.02660.x.
6. Braissant, O., Cailleau, G., Dupraz, C. And Verrecchia, E. P. (2003) 'Bacterially induced mineralization of calcium carbonate in terrestrial environments: the role of exopolysaccharides and amino acids', *Journal of Sedimentary Research,* 73 (3) pp. 485–490. DOI: 10.1306/111302730485.
7. Chauhan, A. and Jindal T. (2020) 'Biochemical and Molecular Methods for Bacterial Identification. In: Microbiological Methods for Environment, Food and Pharmaceutical Analysis', *Springer*.
8. Chen L., Xie A., Jia R., Shen Y., Tang W. and Li C. (2007) 'Influence of *Bacillus subtilis* on the growth of calcium oxalate', *Crystal Research & Technol*ogy, 42 (9), pp. 881–885. DOI: https://doi.org/10.1002/crat.200710933.

9. Chou, C.W., Seagren, E.A., Aydilek, A.H., and Lai, M. (2011) 'Biocalcification of sand through ureolysis', *Journal of Geotechnical and Geoenviromental Engineering.* 137 (12), 1179–1189. DOI:10.1061/(ASCE)GT.1943-5606.0000532.
10. De Muynck, W., Cox, K., De Belie, N. and Verstraete, W (2008), 'Bacterial carbonate precipitation as an alternative surface treatment for concrete', *Construction and Building Material*, 22 (5), pp. 875–885.DOI:10.1016/j.conbuildmat.2006.12.011.
11. Dhami, N.K., Reddy, M.S. and Mukherjee, A. (2013) 'Biomineralization of calcium carbonates and their engineered applications: a review', *Frontiers in Microbiology*, 4. DOI: 10.3389/fmicb.2013.00314.
12. Douglas, S. and Beveridge,T.J., (1998) 'Mineral formation by bacteria in natural microbial communities' . *FEMS Microbiology Ecology* (Oxford Academic) 26 (2), p.p.78-88. DOI: https://doi.org/10.1016/S0168-6496(98)00027-0
13. Dupraz, C., Reid, R.P., Braissant, O., Decho, AW.., Norman, R.S. and Visscher, P.T. (2009) 'Process of carbonate precipitation in modern microbial mats'. *Earth Science Reviews,* 96(3), pp. 41–162. DOI: https://doi.org/10.1016/j.earscirev.2008.10.005
14. Ehrlich, H. L. (1999) 'Microbes as geologic agents: their role in mineral formation', *Geomicrobiology Journal*, **16 (2)**, p.p135–153. DOI: https://doi.org/10.1080/014904599270659
15. Ercole, C., Bozzelli, P., Altieri, F., Cacchio, P. and Del Gallo, M. (2012) 'Calcium carbonate mineralization: involvement of extracellular polymeric materials isolated from calcifying bacteria', *Microscopy Microanalysis,* 18, pp. 829–839. DOI: 10.1017/S1431927612000426
16. Frankel, R.B. and Bazylinski, D. (2003) 'Biologically Induced Mineralization by Bacteria', *Reviews in Mineralogy & Geochemistry – Reviews in Mineralogy and geochemistry,* 54 (1), 95-114. DOI:10.2113/0540095.
17. Jonkers, H.M., Thijssen, A., Muyzer, G., Copurogle, O., and Schlangen, E. (2010). 'Application of bacteria as self-healing agent for the development to sustainable concrete', *Journal of Ecological Engineering*, 36 (2), pp. 230–235. DOI:10.1016/j.ecoleng.2008.12.036
18. Konishi, Y., Tsukiyama, T., Ohno, K., Saitoh, N., Nomura, T. And Nagamine, S. (2006) 'Intracellular recovery of gold by microbial reduction of $AuCl_4^-$ ions using the anaerobic bacterium *Shewanella* algae', *Hydrometallurgy* 81(1), pp. 24–29. DOI: https://doi.org/10.1016/j.hydromet.2005.09.006

19. Lee, C., Lee, H., and Kim, O.B. (2018). 'Biocement Fabrication and Design Application for a Sustainable Urban Area', *Sustainability*.10(11):4079.DOI: https://doi.org/10.3390/su10114079
20. Li, Y.H., Chen, Y.Y.M., and Burne, R.A. (2000) 'Regulation of urease gene expression by *Streptococcus salivarius* growing in biofilms' *Environmental Microbioliogy*, 2(2), pp.169–177. DOI:https://doi.org/10.1046/j.1462-2920.2000.00088.x
21. Lowenstam, H.A and Weiner ,S. (1989) On Biomineralization, *Oxford University Press*, New York
22. Mann, S. (2001) '*Biomineralization: Principles and Concepts in Bioinorganic Materials Chemistry'*. In Compton, R. G., Davies, S. G., and Evans, J. (eds.), *Oxford Chemistry Masters*, New York: Oxford University Press,5, pp216
23. Marques, M.J.F., DiamantioT.C., and Basseguy,R. (2020) 'Assessment of Anticorrosion Properties of Biomineralized Induced Coating formed on Al Alloy in Marine Environment. A new Nature-Inspired Approach for Corrosion Protection', *Open Archive Toulouse Archive Ouverte*, (unpublished)
24. Marvasi, M., Gallagher, K., Casillas-Martínez, L., Pagan, W.C.M., Rodríguez-Santiago, R.E., Vega, G.C. and Visscher, P.T. (2012) 'Importance of B4 Medium in Determining Organomineralization Potential of Bacterial Environmental Isolates', *Geomicrobiology*. 29 (10). DOI: 10.1080/01490451.2011.636145.
25. Marvasi, M., Mastromei, G. and Perito, B. (2020). 'Bacterial Calcium Carbonate Mineralization in situ Strategies for Conservation of Stone Artworks: From Cell Components to Microbial Community'. *Frontiers in Microbiology*, 11(1664-302X). DOI: 10.3389/fmicb.2020.01386
26. Mondal, S and Ghosh, A. (Dey) (2018) 'Investigation into the optimal bacterial concentration for compressive strength enhancement of microbial concrete'. *Construction and Building Materials.* 183, pp. 202-204. DOI: 10.1016/j.conbuildmat.2018.06.176
27. Oppenheimer-shaanan, Y., Sibony-Nevo, O., Bloom-Ackermann, Z., Suissa, R., Steinberg, N., Kartvelishvily, E., Brumfeld, V. and Kolodkin-Gal, I. (2016). 'Spatio-Temporal assembly of functional mineral scaffolds within microbial biofilms', *NPJ Biofilms and Microbiomes*. 2. pp. 15031. DOI: 10.1038/npjbiofilms.2015.31.

28. Perito, B., and Mastromei, G., (2011) 'Molecular Basis of Bacterial Calcium Carbonate Precipitation', *Progress in molecular and sub cellular biology*. 52. pp. 113-39. DOI: 10.1007/978-3-642-21230-7_5.
29. Phillips, A. J., Gerlach, R., Lauchnor, E., Mitchell, A. C., Cunningham, A. B., & Spangler, L. (2013b) 'Engineered applications of ureolytic biomineralization: a review'.*Biofouling*,296, pp.715–733. DOI: https://doi.org/10.1080/08927014.2013.796550
30. Ramachandran, S.K., Ramakrishnana,V. and Bang, S.S. (2001) 'Remediation of Concrete Using Micro-organism',*ACI Material Journal*, 98(1), pp. 3-9. DOI: 10.14359/10154
31. Riding, R. (2000). 'Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms'. *Sedimentology* 47 (1), pp. 179–214. DOI:10.1046/j.1365-3091.2000.00003.x
32. Saldana-Vazquez, R., (2014) 'Intrinsic and extrinsic factors affecting dietary specialization in Neotropical frugivorous bats', *Mammal Review*. 44. DOI:10.1111/mam.12024.
33. Sarikaya, M. (1999). 'Biomimetics: materials fabrication through biology', *Proceedings of National Academy of Science* USA 96 (25), pp.14183–14185 DOI: https://doi.org/10.1073/pnas.96.25.14183
34. Sazanova, K., Kamenetskaya, F., Kamenetskaya, O.V.F., Vlasov, D.Y. and Zelenskaya, M., (2020) 'Carbonate and Oxalate Crystallization by Interaction of Calcite Marble with *bacillus subtilis* and *bacillus subtilis*– *Aspergillusniger* Association', *Crystals*, 10(9):756. DOI: 10.3390/cryst10090756
35. Soon, N. W., Lee, L. M., Khun, T. C., & Ling, H. S., (2013) 'Improvements in engineering properties of soils through microbial-induced calcite precipitation', *KSCE Journal of Civil Engineering*, *17*(4), pp.718–728. DOI: https://doi. org/10.1007/s12205-013-0149-8
36. Tamayo-Figueroa, D.P., Castillo, E. and Brandao, P.F.B., (2019) 'Metal and metalloid immobilization by microbiologically induced carbonates precipitation', *World Journal of Microbiology and Biotechnology*, 35(4), pp. 1-10. DOI: 10.1007/s11274-019-2626-9
37. Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., (2013) 'MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0', *Molecular Biology and Evolution*, 30(12), pp.2725-2729.

38. Tourney, J. And Ngwenya, B. T., (2009) 'Bacteria extracellular polymeric substance (EPS) mediate $CaCO_3$ morphology and polymorphism', *Chemical Geology,* 262 (3-4), pp. 138–146. DOI: 10.1016/j.chemgeo.2009.01.006
39. Varenyam, A., Mukherjee, A., Kumari, D., and Zhang, Q., (2015) 'Biomineralization for sustainable construction – A review of processes and applications', *Earth-Science Reviews*,148(0012-8252),pp.1-17. DOI: https://doi.org/10.1016/j.earscirev.2015.05.008.
40. Weiner, S. and Dove, P. M. (2003) 'An overview of bio mineralization processes and the problem of the vital effect', *Mineralogy and Geochemistry,* 54 (1), pp. 1–29. DOI: 10.2113/0540001
41. Whiffin, V. S., van Paassen, L. A., and Harkes, M. P. (2007). 'Microbial carbonate precipitation as a soil improvement technique', *Geomicrobiology J, 24*(5), pp. 417–423. DOI: https://doi.org/10.1080/01490450701436505
42. Yoshida, N., Higashimura, E., Saeki, Y., (2010) 'Catalytic biomineralization of fluorescent calcite by the thermophilic bacterium *Geobacillus thermoglucosidasius'*. *Applied Environmental Microbiology*, 76, pp. 7322–7327
43. Zhu, T. and Dittrich, M. (2016) 'Carbonate Precipitation through Microbial Activities in Natural Environment, and Their Potential in Biotechnology: A Review', *Frontiers in Bioengineering and Biotechnology*,4. DOI: 10.3389/fbioe.2016.00004